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PULMONARY MYCOSIS OF BIRDS—WITH REPORT OF  
A CASE IN A FLAMINGO.

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### INTRODUCTION.

The association of fungi with many pathological conditions, both in man and the lower animals, has frequently been observed, and among the more important of these affections may be considered pulmonary mycosis. This, as the name indicates, is a disease of the air passages or pulmonary tissue, due to the entrance and subsequent development there of some species of fungus, or mold.


Comparatively speaking, there are but few fungi pathogenic for man and the lower animals, but that there are some is occasionally evidenced by the discovery on autopsy of pneumonias produced by them. These infections of the respiratory apparatus are termed pulmonary aspergillosis when due to one of the various species of *Aspergilleæ*, which, in most instances, is the variety known as the *Aspergillus fumigatus*, or smoky aspergillus. Besides the *Aspergilleæ*, other forms of fungi which may be mentioned as being pathogenic under certain circumstances belong to the *Mucor*, *Oïdium*, and *Oöspora* families. Some of the species variously concerned in the etiology of disease processes are *Aspergillus niger* (van Tieghem, de Barry), *A. glaucus*, *A. candidus* (Robin), *A. fumigatus* (Fresenius), *A. flavus* (de Barry), *A. nidulus* (Eidam), *Eurotium malignum* (Lindt), *Mucor corymbifer* (Lichtheim), *Tricothecium roseum* (Link), and some few others. The parts of the animal body which may be affected are the eye, ear, respiratory apparatus, mouth, liver, kidneys, intestines, lymph glands, genital organs, and the skin.

The pulmonary affection of which this article treats seems to be practically unknown in this country if one is to judge by the number of cases reported, there being only four with which we are familiar, while the other three American reports on aspergillosis refer to the disease affecting the ear and maxillary antrum. But the European literature is fairly rich on the subject, numerous cases having been reported, especially in the past ten years, both in man and animals.

The caseous and pneumonic areas produced often bear a close resemblance to pulmonary actinomycosis, glanders, and tuberculosis. Owing to this similarity, particularly that produced in the experimental disease, to the latter affection, the name pseudo-tuberculosis aspergillina has been suggested by various writers. A large amount of experimental work has been done and the lesions carefully studied, so that the importance of making our case, below mentioned, the subject of a paper may not be apparent; nor is it justified in the light of an economic consideration. However, it is sent forth with the idea that perhaps the disease is more prevalent here than has been reported and that this article may elicit greater interest in the true nature of pathological lesions not bearing the unmistakable imprint of tuberculosis or actinomycosis, which conditions it most closely simulates.

#### HISTORY.

A slight historical review of mycotic affections may be of interest, although it must be admitted that the majority of these earlier articles are incompletely described and the particular species of aspergillus undetermined, or at least only arbitrarily so. This fungus appears to have a predilection for birds, as by far the greatest number of the reported cases refer to the affection in these animals. The first observation was made in 1815, by Mayer and Emmert, who found a fungus in the lungs of a jay. In the following year Jaeger reported the disease in a male swan. From this time numerous instances of cases of mycotic affections have followed one after the other in quick succession in the various domestic and wild birds and in cattle, sheep, horses, dogs, and man. Thus it was reported by Heusinger (1826) in the cavities of the long bones of a white stork; by Thiele (1827), in the lungs of a raven; by Owen (1833), in the bronchi and pulmonary tissue of a flamingo; by Deslongschamps (1841), in the bronchi and air sacs of an eider duck; Rousseau and Serrurier (1841), in the lesions of tuberculosis of a paroquet, and in a pigeon and chicken; by Mueller and Retzius (1842), in the respiratory apparatus of a hawk; by Rayer and Montagne (1842), in the air passages of a tuberculous bullfinch; by Spring (1848), in the abdominal air sacs of a plover; by Robin (1853), in the tuberculous areas of a pheasant; by Fresenius (1858), in the pulmonary tissue of a bustard; by Bouchard (1866), in the lung of a parrot; by Hayem (1872), in lesions simulating caseous pneumonia in ducks; by Leidy (1875) and by Heusinger (1875), in the lungs of flamingos; by Bollinger (1878), Generali (1879), Kitt (1881), and Renon (1893), in pigeons; by Wolff (1883), in the air sacs of gray parrots; by Perroncito (1884), in a chicken; by Schutz (1884), in geese; by Bizard and Pommay (1887), in an ostrich; by Rivolta (1887), in pheasants; by Zschokke (1887), in a swan; by Lignieres and Petit (1898), in a turkey, and by Bland Sutton (1885), who found moldy air sacs as



an exceedingly common condition in the birds dying at the London Zoological Garden, especially among those living in damp places. Pulmonary mycosis has also been noted by other writers in various other birds, and it has been observed to exist at times as an epizootic among poultry. The first case of mycosis in man was reported in 1842, by Hughes Bennett, in the sputum, vomicae, and tubercles of a phthisical patient, although it was not until 1847 that Sluyter definitely demonstrated an *aspergillus* in the lungs of a patient. In 1856 Virchow,<sup>a</sup> who gives references to all previous cases, recorded the presence of aspergillar development in the bronchi and cavities of three tubercular subjects, but he regarded the mycotic invasion as a concomitant occurrence and did not seem to attach much importance to its presence, at least in regard to the genesis of the lesions found. He had also described a condition of the lungs, to which he gave the name of odorless gangrene, which Saxer thinks was due to the invasion of the lung by an *aspergillus*. In 1879 Leber first described a purulent keratitis due to an *aspergillus* infection. Popoff, in 1887, noted in a tuberculous patient, who presented the clinical picture of bronchial asthma, an infection not only of the bronchioles but of the lung tissue with *Aspergillus fumigatus*.

The importance of pulmonary mycosis was indicated in 1890 by Dieulafoy, Chantemesse, and Vidal,<sup>b</sup> who reported their observations and studies of pneumonomycosis as it occurs in a certain class of men in Paris. These men feed thousands of young pigeons daily by taking into their mouths a mixture of grain and water which they force into the mouths of the birds much in the same way that the old pigeons feed their young. It had been a matter of common observation that these men were sufferers from a severe pulmonary disorder; but when their sputum was examined, instead of finding tubercle bacilli, only threads of mycelia were detected. This observation was subsequently confirmed by Renon and other investigators. Until this time it had been held that the presence of fungi in the lung tissue was of secondary importance, but these observations dispelled further argument. Experiments on animals in which they were made to inhale the spores were successful in producing the disease; thus it was that natural infection was proved. With reference to pigeon feeders, it was not ascertained whether they contracted the disease from the pigeon or from spore-contaminated grain until the observation was made in the case of a pigeon which showed mycelia to be developing from a grain of corn that had passed into the bronchiole of the bird. This fact would indicate the probability of infection of these men from a like source.

From observations in England, made by Boyce in 1892 and Arkel

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<sup>a</sup> Virchow's Archiv., Bd. IX, heft 3, pp. 557-593.

<sup>b</sup> Gazette des Hôpitaux, 63, No. 89, 1890, pp. 821-823.

and Hinds in 1896, the disease in man was shown to be of primary origin, but, on the other hand, there can be no doubt that infection will take place more easily in subjects whose lungs are in an already weakened condition.

In 1897<sup>a</sup> Renon published the results of his researches on aspergillosis as seen in birds and animals, and came to the following conclusions:

1. That aspergillosis is a spontaneous disease affecting the bronchi and lungs of birds and animals, and creating in the animals a generalized affection similar to hemorrhagic septicemia; that it develops in eggs in incubation and may contaminate the embryos contained therein.

2. The disease may be transmitted experimentally. The botanical and cultural characters of the fungus and the lesions it provokes are truly specific. In its pathogenic action it bears a strong resemblance to the tubercle bacillus.

3. In man it develops upon the cornea or skin, but has its particular evolution in the respiratory apparatus, creating pulmonary mycosis, resembling tuberculosis, and pulmonary gangrene, but without the fetid odor. It may coexist with tuberculosis. Occasionally it is fatal after the formation of cavities in the lungs. It may invade the bronchial apparatus alone, causing membranous bronchitis of special form and of long duration.

4. In all its manifestations *Aspergillus fumigatus* may play a primary or secondary rôle in both man and animals. It is not, therefore, a simple saprophyte, but a true parasite.

Renon speaks with considerable authority on account of having studied the disease closely in man, besides doing a vast amount of experimental work on animals. His studies of the disease in man showed it to be frequently produced in those whose occupations are of necessity connected with the handling of grain upon which the aspergillus grows or, in fact, any occupation where men are obliged to inhale dust laden with spores. Animals and birds kept in the working rooms of hair-assorting establishments, where rye flour is used as an application to help disentangle the matted hair, became affected and died with aspergillosis of the lungs.

In 1900 Saxer<sup>b</sup> published a historical monograph giving in detail a description of four cases that he had seen in man. In one he had observed a beginning mycosis in croupous pneumonia; in another a fungous development in a large cavity in the lungs of a phthisical patient and consisting of a typical odorless gangrene; in a third healing was taking place, the lung being irregularly nodular and indurated. There is given a full account of his own experimental work, as well as a review of that of others, especially of French authors, and a most complete bibliography of the subject up to that time.

The first observation of mycosis in a domesticated animal was reported by Rivolta in 1857, who observed the fungus in the pharyngeal abscess of a horse. Other cases have been reported by Gotti (1871), who found an aspergillus in auricular catarrh of a dog; by

<sup>a</sup> Étude sur l'aspergillose chez les animaux et chez l'homme, Paris, 1897.

<sup>b</sup> Pneumonomycosis aspergillina, Jena, 1900.

Pech (1876), who observed mycotic pneumonia in seven subjects in a stable where the horses had been fed on moldy hay; by Zurn (1876) in the trachea of a cow; by Martin (1883) in pulmonary abscesses of a horse; by Roeckl (1884) and by Piana (1886) in the lungs of a cow; by Franck (1890) in the greenish caseous nodules in the intestines of cows; by Mazzanti (1891) in the lungs of a lamb; by Goddall (1893) in the ear of a horse; and by Siedamgrotzky (1879), who has seen mycotic pneumonia only as a result of traumatism of the lungs by foreign bodies.

In this country aspergillosis has been observed in but few instances. In addition to the two above-mentioned cases in birds, there have been reported by Theobald (1881) four cases of aspergillosis of the human ear and a subsequent case of a similar affection in 1898. Mackenzie (1893) published a report of finding the *Aspergillus fumigatus* in the maxillary antrum of a woman. In 1887 Osler<sup>a</sup> placed on record the case of a woman who for twelve years had had a severe cough, and in the expectorations mycelial filaments of aspergillar variety were demonstrated. In 1900 Pearson and Ravenel<sup>b</sup> published a review of work done by various investigators on aspergillosis, and gave a very interesting and instructive description of a primary pneumonumycosis of the lung of a cow which had resisted all forms of treatment in efforts to check a progressing emaciation, the nature of which was not understood until the death of the animal.

#### PATHS OF INFECTION.

The liability of the feathered creation to pulmonary aspergillosis is well known, and all species of birds appear to be receptive to the fungus. That the inhalation of spores is by far the most frequent method of infection is admitted by all, and is plainly illustrated by the reported cases. However, Lucet<sup>c</sup> has reported one case of intestinal aspergillosis, Franck has described a similar condition in a cow, Renon noted several cases in rabbits which he produced experimentally by feeding, and Ceni<sup>d</sup> has recently recorded a number of instances in man. Renon and others have also shown that the embryo can become infected through the shell of an egg during incubation. Traumatism has likewise been held accountable for the entrance of the fungus. These latter modes of infection are so infrequent, however, that for all preventive purposes the respiratory tract may be considered as the point of selection of the fungus. The experiments so far conducted have not added materially to our intimate knowledge of the working of the spores of the aspergillus after they are implanted on the bronchial

<sup>a</sup>Trans. of the Path. Soc. of Phila., Vol. XIII, pp. 108-109.

<sup>b</sup>Jour. of Comp. Med. and Vet. Arch., Vol. XXI, No. 8, Aug., 1900, pp. 451-465.

<sup>c</sup>De l'aspergillus fumigatus chez les animaux domestiques et dans les œufs en incubation, Paris, 1897.

<sup>d</sup>Centralbl. fur a. Path. und Path. Anat. Bd. XIV, No. 12, Juli 1903, s. 465-468.

mucosa. The fact that they often produce pneumonias in birds is well established, but just how or why certain species of the *Aspergilleæ* should be the organism most often concerned is another question which remains to be solved. Lately a great deal of work has been done by Ceni and Besta in their investigation into the cause of pellagra in an effort to isolate the toxin which they think is responsible in a great measure for the symptoms and lesions shown. In fact they claim to have isolated a very virulent toxin from the spores of *Aspergillus fumigatus* and *A. flavus*, but thus far they have not given sufficient proof of this statement. Dogs and rabbits inoculated intra-abdominally with quite large doses died within a few hours to several days with tetanic symptoms, and showed on postmortem examination a general hyperemia of all the organs. Their work in this line has not been confirmed; but should it prove to be authentic, it will go a long way in explaining the ease with which the spores of the genus *Aspergilleæ* enter, overcome, and germinate in the tissues. It has been claimed heretofore that the *Aspergillus fumigatus* produced little or no toxin, and that the destructive properties were due to mechanical irritation alone, but the extensive alterations noted in the tissues appear to be entirely too severe to lay to mechanical interference alone. Once having obtained a foothold, the work of tissue destruction is rapid and complete. Infection no doubt in most cases takes place by inhalation of spores, but that many of these never reach very far into the bronchial branches, and that they are thrown out in the bronchial secretions is very likely.

After their implantation on the bronchial mucosa, many of them germinate and come to full maturity. The spores given off from the fruit heads are then disseminated in the bronchioles and cavernous air spaces, where they exist as a further source for new centers of infection. This fructification can be easily demonstrated on postmortem examination by taking scrapings from the mucosa and putting them under the microscope or by making sections of the diseased lung tissue and examining them after staining with suitable aniline stains, when their relation to the mucosa will be demonstrated. Now, whatever energy is expended from the time of germination of the spores until fructification, just that amount of energy is abstracted from the media upon which they are growing, and in the case of a pulmonary infection this energy is drawn from the tissues. Animals and plants do not possess the faculty of assimilating crude nutritive materials direct, but must have them prepared specially. The foods are altered in both cases through the agency of enzymes. It has been shown lately that bacteria and fungi also must have their food so prepared either by intracellular or extracellular enzymes, and it is possible that the intense tissue reaction in cases of pneumonomycosis may be due to some such digestive process. This would be difficult to prove, but it does not



appear at all improbable, and it would account in a measure for the death of the tissues in the pseudotubercular nodules. Numerous experiments have been tried with the various fungi, especially in relation to the best temperatures for their development and fructification, and it has been found that, although a few are able to germinate in the bronchioles, the *Aspergillus fumigatus* is about the only one which develops a vigorous growth there and fructifies, the temperature of the human body seeming to be quite suitable for this species. Most of the other molds develop at a much lower temperature and are therefore usually harmless even if introduced into the lungs. But for the *Aspergillus fumigatus* the lungs act as a veritable propagating house, furnishing a moist, nutrient soil upon which to grow and a congenial warm, moist atmosphere with a sufficient amount of oxygen for its demand to come to complete maturity and for fructification to take place. When the fungous growth is localized in the bronchial mucous membrane, the condition is known as bronchomycosis. It may be that the tissues are able to forestall entrance into their substance and finally the fungi die and recovery takes place. In birds the growth may extend to the air sacs; this condition is then called cytomycosis. Cases of cytomycosis are very rare; and when it does occur, emaciation of the birds is the predominating symptom. When the lung tissue itself is the seat of invasion, the term pneumonomycosis is applied.

Invasion of the lung tissue by the mycelium is the occasion for an intense inflammatory disturbance with positive chemotaxis. However, this tissue reaction seems to offer the most trifling barrier to the parasitic encroachment in such weakly subjects as birds. Generali states that delicate breeds of pigeons are noticeably susceptible to this disease. Fancy breeds of chickens and pheasants and birds in zoological parks that have to be kept in confinement are more predisposed than those running at large. In birds the lesions are at times localized in the mouth, nostrils, or trachea, as well as in the bronchi, lungs, and air sacs; occasionally the liver, kidney, spleen, and peritoneal membrane may show small tubercle-like nodules, and in the center of their caseous contents the fungous filaments may be demonstrated.

#### REPORT OF CASE.

Our subject was that of a flamingo (*Phoenicopterus ruber*) which died at the National Zoological Park, Washington, D. C., on May 15, 1903, and was sent to this laboratory for an investigation as to the cause of its death. The bird was obtained near Las Tunas, on the southern coast of Cuba, and was received on April 25, 1903, by Dr. Frank Baker, superintendent of the National Zoological Park, to whom we are indebted for furnishing us the carcass of the bird. It had

therefore been in this country less than three weeks and was the fourth flamingo to die at the park in a short space of time, the other three not having been subjected to a postmortem examination. In this case the macroscopic appearance of the lungs was somewhat like the gross lesions of an advanced case of tuberculosis, and the carcass of the bird was very much emaciated. On opening the bird the sternum was carefully removed and the heart dissected away from its attachments; this left the lungs in full view. (See Pl. VI.) They presented a mottled appearance and the normal pink color was replaced almost in its entirety by grayish yellow areas from the size of a pinhead to that of a pea. These foci showed a tendency to coalesce, and were not infrequently surrounded by a hemorrhagic zone or area of diffuse or irregular consolidation. In the center of some of the large areas there was a dark pigmentation simulating anthracosis. The bronchial tubes on their lower surface each have an aperture opening into the supra-sternal air sac, and through this opening could be seen a green velvety membranous lining covering the mucous membrane; it was this peculiar appearance that suggested to us the nature of the trouble. An incision was made into the bronchus and a scraping of the material mounted in glycerine was examined with a high-power lens of the microscope. As was suspected, it proved to be masses of mycelia and the sporulating fruitheads of an aspergillus, which was afterwards demonstrated morphologically and by cultural tests to be the *Aspergillus fumigatus*. The macroscopic appearance of the liver, spleen, and kidneys was apparently normal, but the heart was greatly hypertrophied. In the intestines there were several nodules which were examined for the presence of mycelia but without finding any. All of the lung tissue was saved, and many sections showing the intense and very interesting alterations due to the presence of the fungus have been procured. Unfortunately, none of the tissues other than the lungs were preserved for sectioning, so that a description of the microscopic appearance of these organs, as produced by the spontaneous disease, can not be given. Culture media inoculated from the lungs gave an abundant growth of the aspergillus, but contaminated with a growth of an organism which proved to be the bacterium *pyocyanea*. A pure culture of the fungus was obtained on potato and glycerine agar from the spleen and kidney, which indicated an extension of the disease from the lungs. In Allbutt's System of Medicine the statement is made that aspergillosis never becomes generalized, but this probably has reference to the observation of the affection in man. In July, 1903, however, Ceni reported the generalization of the aspergillus infection in twenty-one out of twenty-eight persons affected with pellagra.

Concerning the symptoms of the disease in this particular flamingo very little can be given, as it was in the possession of the Zoological

Park for only twenty days and was evidently infected before its arrival. During this short period it showed a capricious appetite and appeared to be very much dejected, standing about in a listless manner. The plumage was dull and the wings were pendant. Emaciation was not apparent until death occurred.

#### SYMPTOMS IN BIRDS.

In the beginning of the affection there will probably be nothing shown, and it is only after development of the fungus in the bronchi that a difficulty in breathing manifests itself. The birds become listless, mope, and do not follow the rest of the flock. When made to run they soon become exhausted and fall and have great difficulty in breathing. Even when not disturbed they appear very weak and gasp for breath, extending their heads and making movements as if choking. There is great thirst, but a diminution or complete loss of appetite. The birds become rapidly emaciated, the wings are pendant, the eyelids droop, comb and wattles become quite pale, and a general dejected appearance follows. Usually there is an intense diarrhea which weakens the bird very much. In the experimental disease the diarrhea is an accompaniment just as in that of a spontaneous development. The plumage is said to appear ruffled, and the respirations become croupy, even when the disease has not advanced very far; later they are more rapid and a rattling noise can be heard. In the final stages suffocation is threatened.

When the air sacs are affected very few symptoms manifest themselves, though emaciation is marked. As in any similar condition of the lungs, fever is high, and symptoms that would be manifested in pneumonia of fowls would, of course, show here. There is more or less catarrh of the trachea and bronchi, and if these alone were affected there would probably be nothing to attract notice other than symptoms of bronchitis. Bleeding from the nostrils has been observed in man and in animals, and it may be that this would also occasionally be seen in birds. If the air spaces in the bones become affected, lameness with swelling of the joints may result. The duration of the disease is quite variable and death may take place in from one to eight weeks from asphyxia or marasmus. Duration depends a great deal upon the portion of the respiratory apparatus that is affected; if the aspergillar nodules were localized in the mouth, as it is sometimes in pigeons, or in the bones or air sacs, the duration of the disease would, of course, be much longer than if in the bronchi or lung substance.

#### ETIOLOGY.

A short description of the *Aspergillus fumigatus* with a few remarks on its manner of growth will make clearer the microscopical alterations produced by this fungus. It will grow readily and quite satisfactorily on

sterile potatoes, not quite so well on plain agar or on gelatin, which it liquifies, and most slowly on the surface of plain bouillon. The medium in any case should be acid in reaction for its best development. A slice of rye bread placed in a large petri dish, moistened with water, and sterilized has been found to be an excellent medium upon which to grow this fungus, and for the simple study of the manner of growth nothing could be more handy. However, growth on Raulin's fluid is said to be the ideal way to study it in all its phases. This is a complex formula of mineral salts in water, but has the advantage over the solid media in that growth appears to be much more rapid, spore formation taking place within fourteen hours when kept at a temperature of from  $37^{\circ}$  to  $39^{\circ}$  C. Our own experience does not warrant such a statement, as greater difficulty was encountered in obtaining a good growth on this medium than on potato or rye bread. About twelve to eighteen hours after sowing some spores on potatoes, bread, or in the Raulin fluid a white, downy growth occurs which is composed of an interlacing mass of transparent threads, some (known as the thallum) penetrating into the nutrient media, the others forming a downy, felted mat called the aerial mycelia. The aerial filaments give off here and there vertical branches differing from the others in not having internal partitions and which terminate in a hemisphere 8 to  $20\ \mu$  in diameter. (See Pl. VII.) Upon the surface of this hemisphere a number of closely adapted spurs are placed 6 to  $10\ \mu$  in length, known as sterygmata, and, finally, from the ends of these sterygmata are given off rows of radiating conidia, or spores, 2.5 to  $3.5\ \mu$  in diameter, forming what is called the capitulum. These little colorless globular spores are formed by a constricting process, and it is to the dissemination of them in the bronchial tubes that new centers of infection arise. When the aerial hyphæ begin to send off the fruit-bearing shoots, the color begins to change from the white, cottony growth to a blue green (see Pl. X, fig. 2), then to bronze green; finally, in old cultures, to a decided brown color.

The optimum temperature for the *Aspergillus fumigatus* is from  $35^{\circ}$  to  $40^{\circ}$  C. Spores do not form below  $20^{\circ}$  C. They are found in their natural state, widely disseminated, growing upon dead organic matter and on grain, straw, and similar substances. They are very tenacious of life and are able to conform to various conditions of existence. In moldy, stacked hay and in germinating barley the fungus can bring the temperature up to  $60^{\circ}$  C. It is most readily classified by the appearance of the fruiting heads and especially by measurement of the spores, which are from 2.5 to  $3.5\ \mu$  in diameter. Reliance on the color of growths in culture media is unsatisfactory, as it resembles several other species very closely. Botanically it belongs to the family Perisporiaceæ, and order Ascomycetes, so named because they form their spores by partial divisions in the interior of the tubular mother cells. These

spores can withstand a temperature of 65° C. for seven hours, and are destroyed by a 5 per cent solution of carbolic acid or chloride of zinc only after an exposure of twelve hours. They can retain their vitality in a dried condition for many months—in fact, as long as four years—and when placed under suitable conditions will germinate readily. Thus it is seen that they may be a menace to poultry almost as long as they remain in a dried condition about the premises.

#### PATHOLOGICAL ALTERATIONS.

From cultural tests it was shown that the disease in our subject was generalized, although on gross examination the lungs were the only organs apparently affected. The macroscopic examination of these organs disclosed a picture simulating the gross appearance of an advanced case of pulmonary tuberculosis, with the exception that the bronchial tubes were almost completely plugged with a greenish velvety membranous lining.

Before describing the microscopic findings it may be well to mention that the lungs of birds, instead of being freely suspended in the thoracic cavity, are fixed to the dorsal region of the spine and occupy the intercostal hollows, which causes a singular notched appearance to their superior surface. The primary bronchi, after forming a slight dilatation called a vestibule, pass backward to the posterior extremity. They are disposed on the inferior surface of the first third of the lung, where they penetrate, branching as they go, and ending in the air sacs. The primary branches of the bronchial tubes give off secondary branches, and these in their turn give off tertiary branches. These finer branches open upon a dense network of minute, almost naked, blood vessels, through the delicate walls of which oxygen is obtained. The tertiary branches are convoluted, as if by constricting bands, which form imperfect septæ, and it is in these spaces that blood vessels are placed, bringing them in close contact with the air.

In the secondary bronchi and in the cavernous tertiary bronchi of the flamingo much fibrinous exudate is seen. In its substance threads of mycelia penetrate in every direction, often being disposed in a festooned arrangement upon the septal convolutions. In the bronchial divisions not wholly occluded by the croupous exudate are seen the characteristic aspergillar fruitheads in various stages of development, from that of a slight bulging end of the hypha to those giving off their spores. Included within this alveolar exudate are quite a few leucocytes and red blood cells, but their presence is by no means constant. The bronchial mucosa is often eroded and the lining epithelium replaced by a fibrinous coagula or by a membranous material composed of matted mycelial threads from which hyphæ extend into the air space, forming spore-bearing fruitheads, owing to the presence of oxygen. (See Pl. VIII.) The submucous tissue is permeated by

mycelial filaments, and leucocytes and round cells have literally "lined up" in their endeavor to check the invasion of the fungus. However, in this case the effort seems to be futile, as not only the sub-mucous tissues are invaded, but the deeper structures as well. In fact, penetration of the walls of the injected blood vessels can be easily demonstrated. (See Pl. IX, fig. 2.) The resulting thrombi are impregnated also by the mycelial elements. Hemorrhage into the surrounding tissues, due to the weakening and destruction of the continuity of the vessel wall, is a frequent picture in some of the sections studied. The capillary blood vessels nearly all show perivascular aggregations of lymph cells. The various coats of the arterial walls of those vessels in which penetration has taken place are swollen asunder by an abundant sero-fibrinous exudate rich in round cells and leucocytes. Wherever the mycelial threads are found penetrating a new area, there, too, is found a marked leucocytic disturbance, but apparently in most cases this offers but a very ineffective barrier to the encroachment of the fungus. In several sections examined there appeared an occasional circumscribed area with a necrotic center and at its outer margin radiating mycelial filaments resembling clusters of the ray fungus (*actinomyces*). Between this outer zone and the surrounding healthy tissues evidence of tissue defense is manifested by a belt of round cells and new connective tissue elements. This was the nearest approach to tubercle formation in this case, which, however, was far advanced. No giant cells could be made out.

In animals in which the disease was experimentally induced by the injection of the spores into the blood vessels or into the lung substance, miliary lesions resembling tubercular formations were quite noticeable in the lung tissues, and in these an occasional giant cell was discovered. In the lungs of a chicken which was inoculated directly into the lung substance an acute miliary pseudo-tuberculosis was produced, accompanied by intense hemorrhages into the interstitial tissues, as was also the case in intravenous inoculations. In these tubercular nodules penetrating filaments could be made out, but the spores could not be surely demonstrated, or at least differentiated from other cellular elements. Often the bronchial ramifications were the seat of hemorrhage, in which a noteworthy increase in the number of leucocytes could be observed.

In the experiment animals the kidneys showed by far the greatest amount of alteration. In all cases the blood vessels were engorged, their lumen being so distended as to give at first the impression of an interstitial hemorrhage, while extravasated red blood corpuscles were not infrequently observed throughout the parenchyma of the organ. At various locations in both cortical and medullary portions were seen deeply stained areas composed of infiltrated leucocytes and proliferated embryonic cells, the greater part of which were irregular in shape,

especially toward the center of the foci. When the youngest of these tubercles were examined, after treating the sections with caustic potash and staining with aqueous methylene blue, mycelial filaments could be detected in the midst of this pronounced cellular invasion. In tubercles of longer duration these filaments could not be observed, owing to their obscuration, by the infiltration of leucocytes, epithelioid cells, degenerated renal cells and their fragmented nuclei. Multinucleated cells were at times detected in these areas of necrosis. A tendency toward regeneration was occasionally manifested by the appearance of embryonic elements and connective tissue cells arranged concentrically in the periphery of the tubercles. In such instances the surrounding renal tissue appeared normal, but if the lesion was progressive, degeneration of the surrounding cells was indicated by their swollen granular appearance and by their faintly stained or unstained nuclei.

In the liver of the experiment animals there were minute circumscribed or irregular aggregations of cellular elements interposed in the liver lobules between the rows of hepatic cells or involving the latter themselves. A disintegration of the liver cells in these foci was taking place and the nuclei showed more or less fragmentation. Migrated leucocytes were present together with nucleated round cells. These nodules in most cases were not sufficiently degenerated to give the typical appearance of tubercles, but rather suggested the advancing stage of coagulation necrosis. No giant cells were observed. It was with difficulty that filaments could be made out in these foci, but they were finally demonstrated by aqueous methylene blue following caustic potash treatment and by Weigert's fibrin stain. The liver cells immediately around these areas were either normal or exceptionally in the condition of cloudy swelling. There was also occasionally noted slight fatty infiltration of some of the hepatic cells in the immediate neighborhood of the portal spaces.

#### TECHNIQUE.

In the histological study of the above-described lesions the tissues were fixed in gradually ascending strengths of alcohol, cleared in xylol, infiltrated and embedded in paraffin, and cut in serial sections. The stains employed in bringing out the distribution of the fungus and the pathological alterations consisted of hydrochloric-acid carmine counterstained with gentian violet, methylene blue and eosin, lithium-carmin and Weigert's fibrin method, hematoxylin and picro-fuchsin, Gram's stain and eosin, and hematoxylin and eosin, the first three agents mentioned producing the most excellent results. In demonstrating the mycelial elements in tissues treatment with caustic potash solution, followed by aqueous methylene blue, was found highly satisfactory.

## PATHOGENESIS.

In our experiments to ascertain the pathogenicity of the *Aspergillus fumigatus*, chickens, pigeons, rabbits, and guinea pigs were used, the greater proportion of which succumbed to aspergillosis as a result of the injection.

## EXPERIMENTS WITH CHICKENS.

Mature fowls were inoculated intratracheally, intravenously, and intrathoracically. The following case will give an idea of the results obtained by the intravenous method:

On May 28, Chicken No. 12 received in the wing vein 0.5 c. c. of a suspension of spores in a physiological salt solution. It became languid after six hours, refused its food, and died within twenty-four hours. Culture media was inoculated from all the tissues and growths of the aspergillus were obtained from the heart, liver, and spleen, although macroscopically no lesion in any organ could be discerned.

A smaller dose (0.25 c. c.) of a similar suspension of the aspergillus spores when injected into the wing vein of chickens did not produce death until three to five days. In these cases the birds became dull and listless in twenty-four to forty-eight hours, refused to eat, but drank copiously of water, and died within five days from the time of inoculation. On postmortem examination the carcasses appeared to be feverish, the lungs were injected and at times contained diminutive areas of necrosis. The liver and kidneys, and occasionally the spleen, also showed these small foci of degeneration sprinkled throughout their parenchyma. From these tissues the aspergillus was recovered in pure culture. On October 15 a hen was injected intrathoracically with 0.25 c. c. of saline solution of spores. The bird became ill on the following day. Diarrhea set in, accompanied by great thirst, and on the fourth day following death occurred. On postmortem examination the lungs contained isolated and diffused tubercles surrounded by hemorrhagic zones. The liver, spleen, and kidneys likewise contained small pin-pointed foci from which the specific fungus was recovered. The chicken inoculated intratracheally with 0.25 c. c. of a similar suspension on May 28 remained apparently well, and when killed six weeks later no indication of the disease could be detected either macroscopically or microscopically.

## EXPERIMENTS WITH PIGEONS.

On October 17 a pigeon was injected with 0.75 c. c. of a bouillon suspension of spores of the aspergillus into the trachea. This bird died four days later with a local caseous pneumonia, due to the development of spores in the lungs. The infection had extended to the kidneys and peritoneal membranes, causing small yellowish caseous nodules about the size of a pin head and containing masses of mycelial threads.



Pigeon No. 2 was injected into the wing vein with 0.25 c. c. of a suspension of spores in bouillon. Two days later the bird was found dead. The postmortem examination showed the liver and lungs to be very much congested and the spleen soft, swollen, and injected, but no areas of necrosis were observed. Cultures were obtained from the lung and spleen.

Two other pigeons were fed on three successive days with a bouillon suspension of spores amounting to 10 c. c. daily. This was placed on their grain and was consumed without causing them any apparent inconvenience. When chloroformed eight weeks after their exposure no alterations were discernible and media inoculated with juices of the tissues remained sterile.

#### EXPERIMENTS WITH RABBITS.

Rabbits Nos. 1007 and 1008 were inoculated intraabdominally each with 0.5 c. c. of a saline suspension of spores from a seven-months-old potato culture of *Aspergillus fumigatus*. The former animal appeared well and retained its appetite until the seventh day, when it was observed to be depressed and refused to eat. The animal gradually grew worse and died on the ninth day after becoming so weakened as to lie prostrate for five hours before its death. Postmortem examination showed a well-marked case of aspergillosis. The lungs were invaded with three foci in the principal lobe of the left lung, and four nodules on the apex of the right lung. The heart and pericardium were apparently normal. The peritoneal surface of the diaphragm was sprinkled with small transparent glistening nodules varying in size from a mere speck to a celery seed. The liver contained numerous pin-point areas of necrosis. The spleen and kidneys showed numerous well-scattered, elevated, and circumscribed nodules strongly suggestive of tuberculosis. The pancreas was likewise covered with little pin-point areas of necrosis. The right testicle showed three irregular or raised nodules on the tunica vaginalis. The omentum, mesentery, parietal peritoneum, and serous lining of the intestines were thickly sprinkled with minute transparent dew-like elevations. The most interesting feature connected with this autopsy was the finding of several circumscribed yellowish white foci about the size of a mustard seed in the adductor muscles of the left hind leg. Culture media inoculated with the affected organs gave luxuriant growths of the specific fungus.

Rabbit 1008 failed to show any marked symptoms for the first two weeks after inoculation. It then began to lose weight, and on the twenty-fifth day was chloroformed. The postmortem examination showed an involvement of the liver, spleen, kidneys, and abdominal serous membranes, as in the preceding rabbit, but to a less extent. The organs of the thoracic cavity were apparently normal.

#### DESCRIPTION OF PLATE VI.

Pneumonomycosis in the lung of a flamingo caused by the *Aspergillus fumigatus*. (Natural size.)

#### DESCRIPTION OF PLATE VII.

Cover-glass preparation of the *Aspergillus fumigatus*, forty-eight hours old, taken from the surface of dextrose bouillon and mounted in glycerine. Enlarged twice the original when drawn opposite base of stand with the camera lucida, using Zeiss 8 mm. objective and No. 4 compensating ocular.

#### DESCRIPTION OF PLATE VIII.

Section from lung of flamingo stained with hydrochloric-acid carmine, and gentian violet. Camera-lucida drawing made with Zeiss 8 mm. objective and No. 4 compensating ocular at the base of stand. Notice the fruit heads and free spores in the bronchus, the lining membrane of which is completely eroded and replaced by matted mycelial threads of the fungus.

#### DESCRIPTION OF PLATE IX.

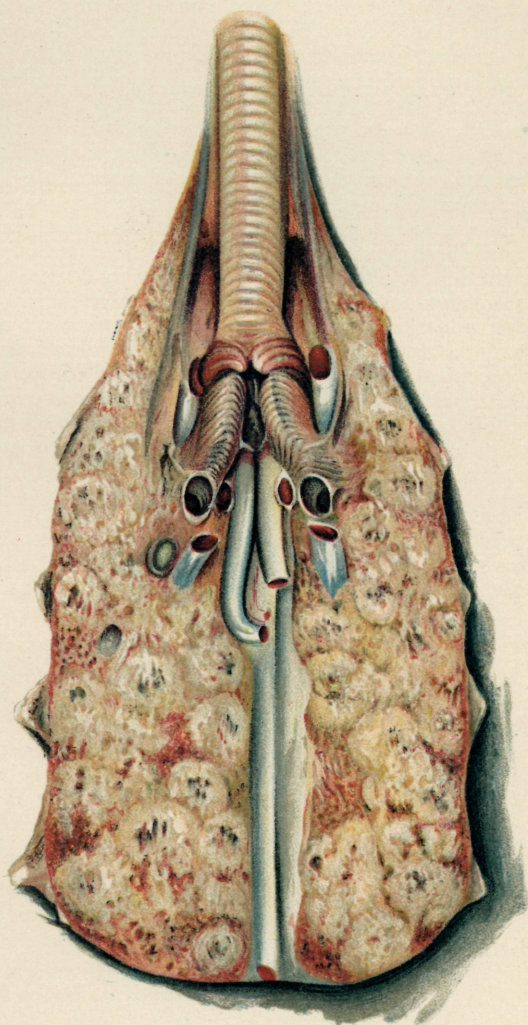
Fig. 1. Section from lung of flamingo stained with methylene blue and eosin. Camera lucida drawing made with Zeiss 8 mm. objective and No. 4 compensating ocular at the base of stand. Notice the marked absence of cellular exudate in the bronchus. Fruit heads and free spores are shown in the air space, a fibrinous coagulum along its wall, and mycelial filaments penetrating the pulmonary tissue, which shows pronounced cellular invasion.

Fig. 2. Section from another portion of the same lung stained with methylene blue and eosin. Camera lucida drawing made with Zeiss 16 mm. objective and No. 6 compensating ocular at stage level. Mycelial filaments are observed penetrating the walls of the blood vessel, producing a thrombus, in which many leucocytes and fungous elements may be detected.

#### DESCRIPTION OF PLATE X.

Fig. 1. Six-day-old culture of *Aspergillus fumigatus* on potato, showing the vigorous character of growth and its bluish green tint at this stage of development. (Reduced one-fourth.)

Fig. 2. Aspergillar tubercles in the kidneys and suprarenal capsules of rabbit No. 850, which died on the tenth day following the intravenous injection of the spores of the fungus. (Natural size.)



PNEUMONORMYCOSIS, LUNG OF FLAMINGO.



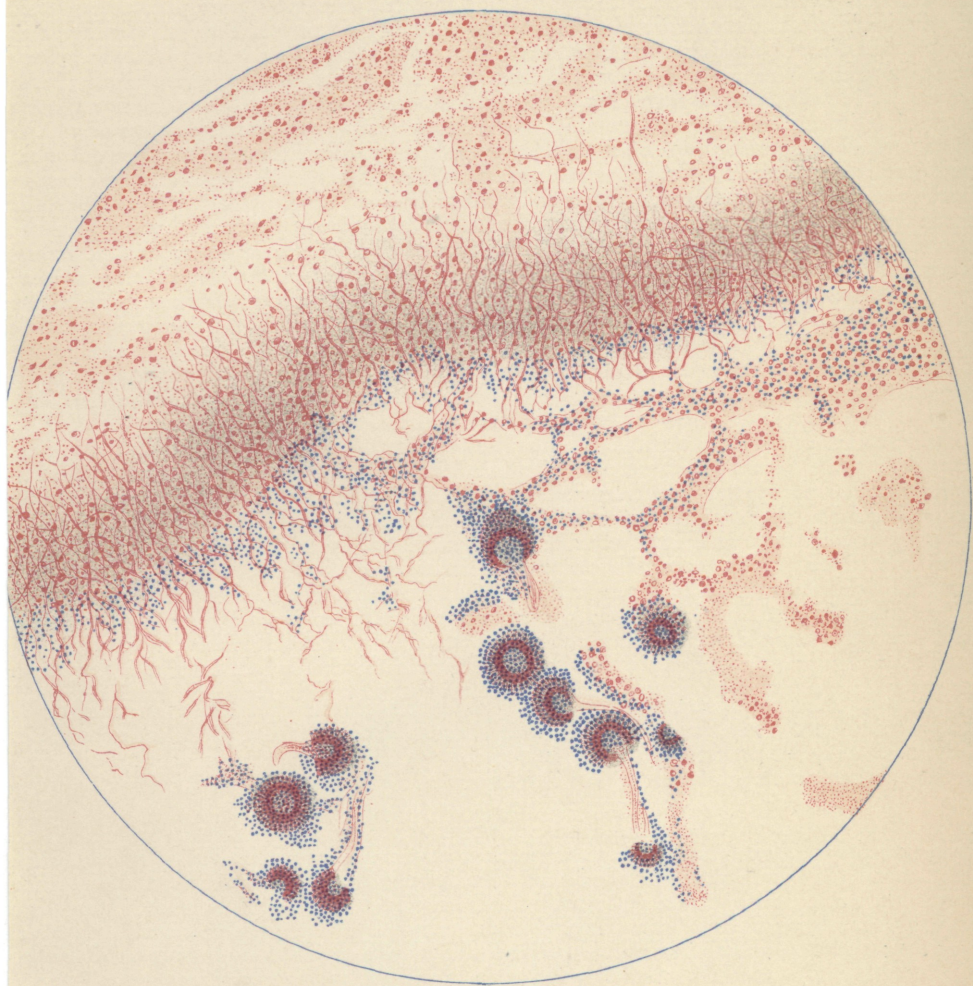


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ASPERGILLUS FUMIGATUS.



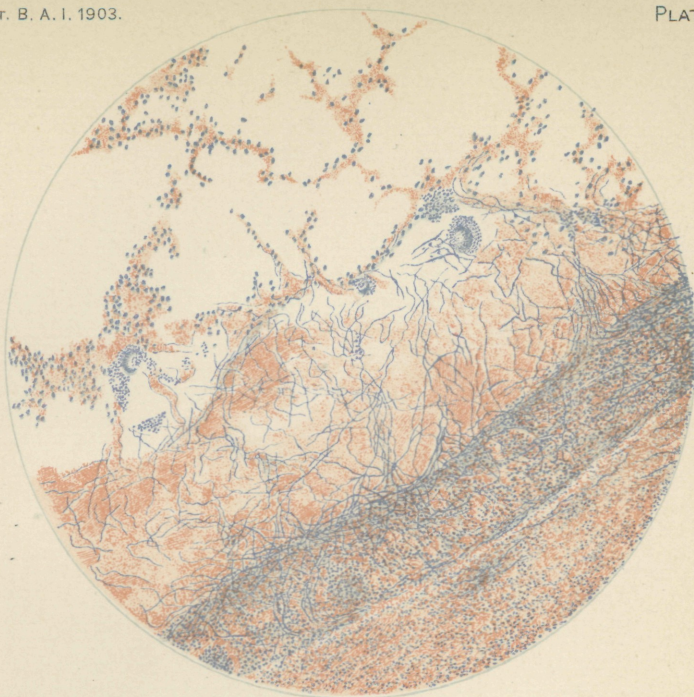


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ASPERGILLUS FUMIGATUS IN LUNG OF FLAMINGO.





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SECTIONS OF NODULES IN LUNG OF FLAMINGO.

FIG. 1.

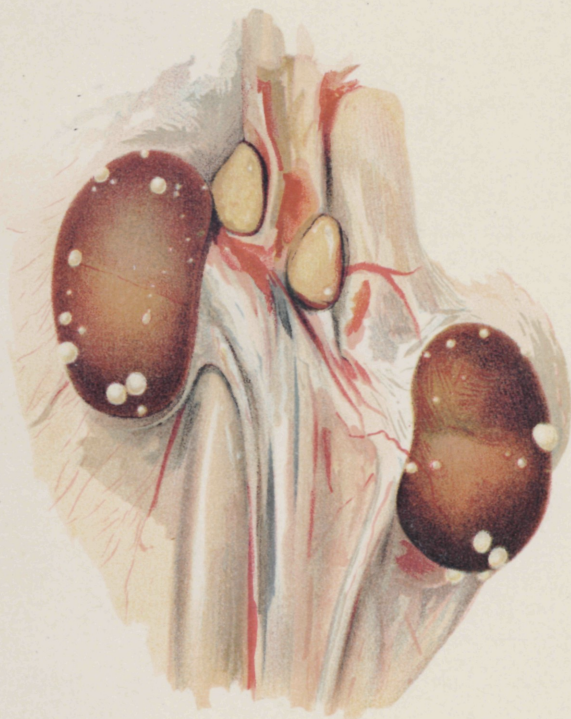


FIG. 2.

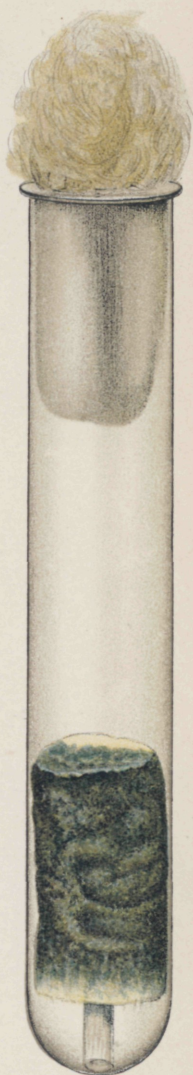


FIG. 1.—EXPERIMENTAL ASPERGILLOSIS IN RABBIT.

FIG. 2.—POTATO CULTURE OF *ASPERGILLUS FUMIGATUS*.